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54 Expectorants comprising N-acetylneuraminic acid and its salts.

57 An expectorant capable of acting on the rheological properties of sputum and capable of directly promoting the ciliary motion. The expectorant contains N-acetylneuraminic acid or a pharmaceutically active salt thereof.

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Title of the Invention

EXPECTORANT

TITLE MODIFIED
see front page 10. Sep. 1985

Background of the Invention

(1) Field of the Invention

5 The present invention relates to an expectorant and a
method of removing sputum by using the expectorant. More
particularly, the invention is concerned with an expectorant
made of N-acetylneuraminic acid and its pharmaceutically
active salt having an effect of directly promoting ciliary
10 motion while acting on the rheological property of sputum, as
well as to a method of removing sputum by such an expectorant.

(2) Description of the Related Art Including Information

15 Viscous secretion exists in the airway of the human
body. The secretion has an important role in imparting
suitable temperature and humidity to inhaled air. The matter
generally referred to as "sputum" is constituted mainly by
this secretion.

20 When its amount is moderate, the secretion in the
airway is unconsciously swallowed or expelled with the breath,
but usually is never expectorated. Thus, any expectoration
suggests that there is something extraordinary in the
respiratory system. On the other hand, accumulation of sputum
to be expectorated in the airway is liable to cause an
25 infection via the airway. From this point of view, the
removal of sputum is a matter of great significance in the
medical treatment of patients who suffer from a disease in the
airway.

Sputum is often very viscous and sticky so that expectoration causes the patient pain. In order to facilitate expectoration, therefore, a medicine which is referred to as "expectorant" has been used. This medicine is given in a general dosage orally or by injection. The expectorant thus administered serves to remove sputum by diluting the sputum through an increase in secretion by the mucosa of the airway, promotion of separation from the mucosa and enhancement of ciliary beat. This, however, involves various clinical problems concerning mechanism and effect.

The present inventors have therefore made intensive studies and found that sialic acids may serve as

an effective expectorant which can be used locally.

This invention has been accomplished through a study on the sputum-removing effect offered by N-acetylneuraminic acid and its salt, among the sialic acids.

Summary of the Invention:

Accordingly, an object of the invention is to provide an expectorant mainly composed of N-acetylneuraminic acid or its salt, as well as a method of removing sputum by local dosage of the expectorant which acts on the rheological property of sputum and which directly promotes ciliary beat so as to facilitate the expectoration.

Brief Description of the Drawings:

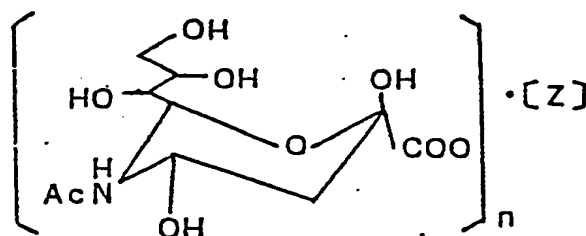
Figs. 1 and 2 are illustrations of the effect of N-acetylneuraminic acid on the rotation of ciliated cells; and

Figs. 3 and 4 are illustrations of ^{the} effect of terbutaline and acetylcholine on the rotation of ciliated cells.

Brief Summary of the Invention:

(A) Effective Components

The expectorant of the invention contains, as an effective component, an N-acetylneuraminic acid or its salt which is expressed by the following general formula:



On condition of $n = 1$, Z represents hydrogen, lithium, potassium, sodium, ammonium, organic ammonium or an organic amine, whereas, on condition of $n = 2$, Z represents calcium, barium or magnesium. The N-acetylneuraminic acid itself is a known compound, and its salt can be obtained by neutralizing this acid with a hydroxide or carbonate of an alkali metal or alkali earth metal and separating the corresponding alkali metal salt or alkali earth metal salt from the system.

According to the invention, this compound is used in such a form as to permit the use of a known local administration method such as inhalation of the pulverized compound from an aerosol.

5

(B) Mechanism

The effect of N-acetylneuraminic acid and its salt as an expectorant is attributable to the following two functions: namely, physiological change for easier expectoration by acting on the rheological property of ^{the} expectorant such as its fluidity, yield value, spinnability, stickiness, stress relaxing time and so forth, and promotion of sputum transportation by cilia through direct activation of ciliary beat of the cilia in the airway. These functions have been confirmed through an experiment in which the rotary motion of a cilium group before the application of N-acetylneuraminic acid or its salt was compared with that after the application, using cilium cells in the human airway, as well as through an experiment in which the sputum conveying property of cilia of frog palatine mucosa in the state after the application of the N-acetylneuraminic acid or its salt was compared with that before the application.

(C) Form of Preparation

In clinical use, the expectorant of the invention is used in the form of, for example, the following preparation.

Example of Preparation:

As the first step, N-acetylneuraminic acid or its salt is well ground and mashed in an agate mortar to reduce it to a fine powder of a grain size ranging between 1 and 20 microns. Lactose is then gradually mixed with the fine powder and diluted 10 to 20 fold. Then, 20 to 40 mg of the mixture is put in a capsule or envelope by a known method. Preferably, a capsule is used for powdered aerosol, and an envelope is used for liquid aerosol.

(D) Method of Administration

Whether the expectorant is used as a powdered aerosol or liquid aerosol is determined on the basis of the symptoms of the patient. The dose is usually 0.5 to 5 mg/time. The expectorant should be administered at least two times a day but this may be changed if the symptoms warrant it.

The results of pharmacodynamic tests and acute toxicity tests showed that the number of dosage per day need not be limited.

The expectorant is administered, when used in the form of a powdered aerosol, by means of a powder spray such as an insufflator or nebulizer, and when used as a liquid aerosol, by a liquid atomizer such as a nebulizer.

The effect of the invention can be summarized as follows: the expectorant of the invention exhibits a superior sputum-removing effect when administered locally. Namely, it changes the property of sputum to ease expectoration by acting

on the rheological property of sputum, e.g. yield value and viscosity. On the other hand, it directly activates the ciliated cells to promote expectoration by ciliary beat.

5 Description of the Preferred Embodiments:

Description will be now made hereinunder as to the way of synthesis of the compound of the invention, as well as the effect of the same, through preferred examples.

10 Example 1

Method of Synthesizing Ammonium Salt of N-Acetylneuraminic Acid

12 g of N-acetylneuraminic acid was dissolved in 150 ml of water to form an aqueous solution into which was stirred a saturated aqueous solution of ammonium until the solution becomes neutral (pH 6.7). The reaction solution was spontaneously filtrated and the residual was freeze-dried (one day) to form 13.2 g of white crystals.

20 Melting Point: about 150°C decomposed,

Qualitative Reaction of Ammonium Salt:

According to General Testing Method of the Pharmacopeia of Japan, 10th Edition

25 An excess amount of sodium hydroxide reagent was added to the ammonium salt and the mixture was heated. The mixture gave off an odor of ammonium and the fume changed a red litmus paper blue.

(Elementary Analysis)

N-acetylneuraminic acid ammonium 1-hydrate



Calculated value

Measured value

| | | | | |
|---|----|--------|-----|---------|
| 5 | C: | 38.48% | C: | 38.11% |
| | H: | 7.05% | H: | 6.92% |
| | N: | 8.16% | N: | 8.17% |
| | O: | 46.60% | (O: | 46.80%) |

10 Example 2:

Method of synthesizing Calcium Salt of N-Acetylneuraminic Acid

200 ml of distilled water was added to a mixture of 10.1 g (0.0323 mole) of ^{N-acetylneuraminic acid} and 1.162 g (0.0162 mole) of calcium carbonate. The solution was stirred for 2 hours at room temperature until the calcium carbonate was completely dissolved (pH 6.55). The solution was filtered and the deposit was subjected to freeze-drying and decompression drying to form 11.0 g of colorless needle crystals.

Melting Point: 140° or higher (decomposed)

Qualitative Test of Calcium Salt

20 According to General Testing Method of Pharmacopeia of Japan 10th Edition

(1) A yellowish red was observed in a flame reaction test of the calcium salt.

25 (2) A white precipitate was observed when ^{an} ammonium carbonate reagent was added to the calcium salt.

(Rlv^{KBr}_{MAXcm}⁻¹) 3600 - 3000, 1620
1560

(NMR(D₂O) ppm) 1.59 - 2.42 (m)
2.05(s), 3.71 (s)
5 3.99(s)

Salts of lithium, potassium, sodium, organic ammonium, barium and magnesium of N-acetylneuraminic acid were prepared in the same way as that described above. Namely, the N-acetylneuraminic acid was neutralized with hydroxides or
10 carbonates of these elements, followed by freeze-drying, thus obtaining salts of ^{the} N-acetylneuraminic acid.

Example 3:

Effect of N-acetylneuraminic acid and Calcium Salt of N-
15 acetylneuramic acid on Ciliary of Palatine Mucosa of Frog

(1) Test Animal

Bull frogs (furnished by Saitama Test Animal) weighing 300 to 450 g were used regardless of sex.

20 (2) Test Material

N-acetylneuraminic acid and ^{the} calcium salt of N-acetylneuraminic acid (provided by Kanto Ishiseiyaku Kabushiki Kaisha), as well as acetylcholine chloride (furnished by Sigma Kabushiki Kaisha) were used as test substances.

(3) Test Method

Mucociliary transport was measured by the particle transport method. After decapitation of a frog, the surface of the palatine mucosa was separated with minimized damage and

5 fixed to a cork plate in a Petri dish which was then filled with 20 ml of Ringer's solution (trishydrochloric acid). The palatine mucosa was left in this state for 30 minutes before the commencement of measurement. For measurement, the Ringer's solution was removed and fine pieces of cork (capable
10 of passing pharmaceutical sieve No. 5 but not capable of passing No. 6) were placed on the surface of the palatine mucosa, and the time (in seconds) required for the cork piece to move 1 cm was measured. The duration of movement, i.e., moving speed, was measured also in the state immediately after
15 the removal of the Ringer's solution or pharmaceutical solution.

(4) Result

20 It was confirmed that N-acetylneuraminic acid and its calcium salt causes an acceleration of the ciliary movement of palatine mucosa in a certain dose-dependency, as will be seen from Tables 1 and 3.

This result was substantially equal to that produced by acetylcholine (control) at concentrations of 10^{-5} and 10^{-4}
25 g/mol, as will be seen from Table 2.

(5) Judgement

N-acetylneuraminic acid and its calcium salt were confirmed as producing substantially the same ciliary movement promoting effect as the acetylcholine which has been known as being effective in the promotion of ciliary movement. N-acetylneuraminic acid and its calcium salt, therefore, are expected to facilitate expectoration. The acetylcholine, however, cannot be used as an expectorant because it causes undesirable effects such as bronchoconstriction and crinogenic action, although it promotes ciliary action. In contrast, N-acetylneuraminic acid and its calcium salt can be used as an expectorant because they effectively promote ciliary action, without being accompanied by the detrimental effects produced by acetylcholine.

Table 1

Effect of N-acetylneuraminic Acid on Ciliary Movement

| Drug g/ml | | Movement (sec/cm) Frog Number | | | | Acceleration (%) * Frog Number | | | |
|---------------------------------|------------------|-------------------------------------|------|------|------|--------------------------------------|------|------|------|
| | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Control | - | 32.0 | 32.7 | 40.3 | 36.0 | 0 | 0 | 0 | 0 |
| N-acetyl- neuraminic acid | 10 ⁻⁵ | 21.3 | 27.3 | 28.7 | 24.7 | 33.4 | 16.5 | 28.2 | 31.4 |
| | 10 ⁻⁴ | 17.3 | 22.0 | 20.7 | 22.0 | 45.9 | 32.7 | 48.2 | 38.9 |

$$\text{Acceleration (\%)} = \left(1 - \frac{\text{time in drug}}{\text{time in control}}\right) \times 100$$

Table 2

Effect of Acetylcholine on Ciliary Movement

| Drug g/ml | | Movement (sec/cm) Frog Number | | | Acceleraiton (%)* Frog Number | | |
|--------------------|------------------|-------------------------------------|------|------|-------------------------------------|------|------|
| | | 1 | 2 | 3 | 1 | 2 | 3 |
| control | - | 45.0 | 37.0 | 39.3 | 0 | 0 | 0 |
| acetyl- choline | 10 ⁻⁵ | 27.0 | 29.7 | 26.3 | 40.0 | 19.7 | 33.1 |
| | 10 ⁻⁴ | 21.0 | 21.7 | 20.7 | 53.3 | 41.4 | 47.3 |

10 Acceleration (%) = $(1 - \frac{\text{time in drug}}{\text{time in control}}) \times 100$

Table 3

Effect of Calcium Salt of N-Acetylneuraminic Acid on Ciliary Movement

| Drug g/ml | | Movement (sec/cm) Frog Number | | | Acceleraiton (%)* Frog Number | | |
|---------------------------------|------------------|-------------------------------------|------|------|-------------------------------------|------|------|
| | | 1 | 2 | 3 | 1 | 2 | 3 |
| control | - | 37.7 | 40.0 | 37.0 | 0 | 0 | 0 |
| calcium salt of | 10 ⁻⁵ | 25.0 | 29.3 | 24.3 | 33.7 | 28.0 | 34.2 |
| N-acetyl- neuraminic acid | 10 ⁻⁴ | 23.0 | 29.0 | 19.7 | 39.0 | 28.7 | 46.8 |

20 Acceleration (%) = $(1 - \frac{\text{time in drug}}{\text{time in control}}) \times 100$

Example 4:

Effect of N-acetylneuraminic Acid and Its Calcium Salt on
Rotation of Ciliated Cell

(Object)

5 Using ciliated cells from ^{the}human airway and frog
palate, rotation of cell groups due to ciliary beat was
observed before and after application of N-acetylneuraminic
acid and ^{the}calcium salt of N-acetylneuraminic acid, in order to
confirm the effect of these agents on the ciliated cells.

10

(Method)

Human ciliated cells were collected from a normal
trachea by means of a branchoscope, the ciliated cells being
separated by rubbing the bronchus while observing through the
15 branchoscope, while the collection of frog ciliated cells was
conducted by rubbing the palatine mucosa, without being
specially careful. The cilium cells thus obtained were
floated in Medium 199 (prepared by Gibco Company) and groups
of ciliated cells, each consisting of several cells exhibiting
20 rotary motion due to ciliary motion, were sucked up by a
microsyringe in such a manner as to avoid damage to such
cells, using observation through a phase contrast microscope.
The groups of cells were transferred to a cover slip and one
drop of chicken plasma (prepared by Difco Company) and one
25 drop of 50% chicken embryo extract were added to the cells,
thus forming clots. The rotary motion in the clots was
recorded by means of a video recorder and the number of

rotations per minute was measured. The agents were dissolved in the Medium 199 and were injected through a tube attached to a rose chamber. The states of rotary motion 5 minutes, 10 minutes and 15 minutes after the injection were recorded by means of a video recorder, and the number of revolutions was measured.

(Result)

With application of N-acetylneuraminic acid (10^{-4} to 10^{-1} mg/ml), the rotary motion of the cilium cells is gradually accelerated starting immediately after the application. 5 minutes, 10 minutes and 15 minutes after the application, plateaus were observed at application rates of 10^{-3} to 10^{-2} mg/ml. In these plateaus, the speed in revolutions per minute was about 25% higher than that in the Medium 199, as will be seen from Figs. 1 and 2. It was confirmed also that the rotary motion of the cilium cells is increased by application of 10^{-3} to 3×10^{-2} mg/ml of terbutaline (5-[2-[(1,1-dimethylethyl) amino]-1-hydroxyethyl]-1,3-benzenediol), in a certain dependency to the dose. The effect was particularly remarkable 5 minutes after the application, as can be seen from Fig. 3.

It was confirmed also that the speed of rotary motion of the cilium cells is increased by the addition of 10^{-3} mg/ml and 3×10^{-3} mg/ml of acetylcholine. Speed peaks of the rotary motion were observed about 10 minutes and 5 minutes after the application when the amounts of addition were

respectively, 10^{-3} mg/ml and 3×10^{-3} mg/ml. In both cases, the rotary motion stopped 13 minutes after the application.

(Judgement)

5 It was confirmed that terbutalin and acetylcholine, which are known as being effective in promoting ciliary beat, promote also the rotary motion of the cilium cells. N-acetylneuraminic acid was confirmed as also being effective in the promotion of the rotary motion of ciliated cells. It is
10 considered that the rotary motion of the ciliated cells is transmitted into ciliary beat, thereby facilitating the expectoration. This clearly shows that N-acetylneuraminic acid will be effective as an expectorant.

15 Example 5:

Effect on N-acetylneuraminic Acid on Sputum

(Object)

 Mucociliary transport rate (hereinunder referred to as MTR), i.e. the rate of transport of sputum by cilia, was
20 measured using frog palatine mucosa. At the same time, physicochemical properties of the sputum before and after application of N-acetylneuraminic acid were compared.

(Method)

25 The sputum used for the specimens was collected from 12 samples of patients suffering from chronic obstructive lung disease. N-acetylneuraminic acid was added to the specimen

sputum in the ratios of 5 mg/ml sputum and 1 mg/ml sputum, and the specimen was left for 1 hour at 40C before measurement. Sputum which had not been treated by N-acetylneuraminic acid was used as a comparison example. A palatine mucosa obtained from a decapitated frog head was kept at constant temperature and humidity until the mucus liquid of the frog itself depleted. The specimen sputum was placed on the dried palatine mucosa, and the speed of movement of the sputum was observed through a noumenal microscope, thus measuring the MTR. The pH value of the sputum was measured by means of an electronic model HM-Sap pH meter, while the spinnability was measured by a device developed by Nagaoka and Yamanaka (disclosed in Transactions of 2nd Symposium of Cough and sputum, published July 20, 1979). The yield value was measured by Dulfano's double-tube method which is shown in the American Review of Respiratory Diseases, Vol. 104, p88, 1971.

(Result)

As shown in Table 4, five out of six samples treated with 1 mg/ml of N-acetylneuraminic acid showed a reduction in pH value ($\text{pH } 7.31 \pm 0.40$), while the remaining one sample showed a slight increase in pH value. Increase in the yield value was observed in three samples, while three samples showed a reduction. The yield value was unchanged in the remaining one sample. As to the spinnability, five samples showed a reduction, while one sample showed a slight increase. The samples which showed reduction in the spinnability exhibited a tendency to increased MTR.

Table 4

Effect of N-acetylneuraminic Acid or Sputum

5

| No. | Treatment | Apperance | Amount ml | pH | Yield value mmH ₂ O | Spin- nability mm | MTR mm/min |
|-----|-----------|-----------|--------------|--------------|--------------------------------------|-------------------------|---------------|
| 1 | B* A** | M | 2 | 7.72 7.35 | 3.7 11.5 | 55 19 | 19 38 |
| 2 | B A | M | 2 | 7.52 7.62 | 0.1 0.1 | 10 3 | 8 19 |
| 3 | B A | P | 2 | 7.98 7.20 | 2.7 0.7 | 5 2 | 20 27 |
| 4 | B A | M | 2 | 6.98 6.84 | 1.9 3.2 | 22 12 | 25 29 |
| 5 | B A | M | 2 | 7.24 6.94 | 2.2 3.1 | 18 16 | 18 30 |
| 6 | B A | M | 2 | 8.16 7.88 | 14.3 7.5 | 22 26 | 15 18 |

10

- 15 B* : Before treatment with N-acetylneuraminic acid (2 mg)
 A** : After treatment with N-acetylneuraminic acid (2 mg)
 P : Pus sputum
 M : Viscous sputum

20 Example 6:

Acute Toxicity Test of N-acetylneuraminic Acid

A test was conducted to examine the acute toxicity of N-acetylneuraminic acid, using mice and rats, by means of intravenous injection, subcutaneous injection, per os
 25 inhalation.

(1) Test Animals

ICR mice

6 weeks old

SD rats

6 weeks old (7 weeks old in case
of inhalation)

5

(2) Application Density

13 to 15% (W/V) dissolved in distilled water

(3) Number of Test Animals per Group

10

10

(4) Observation Period

14 days

15

(5) Method of Calculation of LD₅₀

By Litchfield-Wilcoxon method

The result of the test is shown in Table 5 below.

Table 5

LD₅₀ (mg/kg)

| Route | mice | | rats | |
|-------------------|---------------------|---------------------|-------------------------|-------------------------|
| | M | F | M | F |
| intrav- enous | 1700 (1441-2008) | 2500 (2137-2925) | 1870 (1748-2001) | 1880 (1741-2030) |
| subcutan- eous | > 5000 | > 5000 | > 5000 | > 5000 |
| per-os | > 5000 | > 5000 | > 5000 | > 5000 |
| inhalation | -- | - | > 4.33 g/m ³ | > 4.33 g/m ³ |

Inhalation was conducted by 1-hr exposure to mist. Values in parentheses show 95% reliability level.

Example 7:

Synthesis of Sodium Salt of N-Acetylneuraminic acid

A small amount of activated carbon was added to an aqueous solution of N-acetylneuraminic acid and, under a flow of nitrogen gas, 1N sodium hydroxide was added to obtain a pH value of pH 7.3 to 7.8. The reacted solution was filtered using a filter of 0.2 μ m and the filtrate was freeze-dried, forming a colorless powder of sodium salt of N-acetylneuraminic acid.

0177783

1R 3400 cm^{-1} , 2937 cm^{-1}

1660 to 1620 cm^{-1} , 1560 cm^{-1}

decomposition point

about 140 - 185°C

(decomposition with foaming)

5

^1H -400MHz (D_2O)

1,807 (dd, H3ax)

2,205 (dd, H3eq)

4,022 (m, H4)

3,901 (t, H5)

10

3,973 (d, H6)

3,501 (d, H7)

3,747 (m, H8)

3,598 (dd, H9')

3,831 (dd, H9)

2,041 (s, Ac-5)

15

Example 8:

Effect of Sodium Salt of N-Acetylneuraminic Acid on Ciliary Movement of Palatine Mucosa of Frog

20

Using the sodium salt of N-acetylneuraminic acid obtained in Example 7, a test was conducted to confirm the above-mentioned effect in accordance with the same testing method as that of Example 1. The result of this test is shown in Table 6.

25

From Table 6, it will be seen that the sodium salt of N-acetylneuraminic acid caused acceleration of the MTR of the frog palatine mucosa in a certain dependency on the dose. The result was substantially equivalent to that of the acetylcholine (comparison agent) at a concentration of 10^{-4}

mg/ml. This suggests that the sodium salt of N-acetylneuraminic acid produces an effect similar to that produced by the above-described N-acetylneuraminic acid and its calcium salt.

5

Table 6

Effect of Sodium Salt of N-Acetylneuraminic Acid on Mucociliary Transport

Acceleration: mean value \pm standard deviation (%*)

10

| agent | amount of addition (mg/ml) | N | time after applied (min) | | |
|--|-------------------------------------|---|--------------------------|------------------|-----------------|
| | | | 5 | 10 | 15 |
| control | - | 8 | -1.6 \pm 6.3 | -11.9 \pm 10.8 | -7.4 \pm 9.8 |
| 15 sodium salt of N-acetyl- neuraminic acid | 10 ⁻⁴ | 8 | 2.6 \pm 22.2 | 8.5 \pm 21.9 | 7.1 \pm 27.2 |
| | 10 ⁻³ | | 16.2 \pm 19.5 | 18.9 \pm 22.1 | 17.7 \pm 34.0 |
| | 10 ⁻² | | 20.8 \pm 25.5 | 18.0 \pm 29.7 | 5.7 \pm 24.9 |
| | 10 ⁻¹ | | 10.7 \pm 19.2 | 8.5 \pm 23.4 | 7.4 \pm 24.7 |
| 20 acetylcholine | 10 ⁻⁴ | 8 | 3.0 \pm 7.6 | 3.8 \pm 15.5 | -5.5 \pm 10.0 |
| | 10 ⁻³ | | 12.3 \pm 8.5 | 19.9 \pm 16.8 | 13.9 \pm 20.5 |
| | 10 ⁻² | | 17.5 \pm 14.3 | 18.8 \pm 21.1 | 24.9 \pm 25.6 |
| | 10 ⁻¹ | | 22.8 \pm 26.1 | 31.0 \pm 24.9 | 30.5 \pm 19.5 |

$$\text{Acceleration (\%)} = \left(\frac{\text{Transport Rate in Drug}}{\text{Transport Rate in Control}} - 1 \right) \times 100$$

25 Over 20.0% is judged as significant.

Example 9:

Effect of Sodium Salt of N-Acetylneuraminic Acid on Rotary Motion of Ciliated Cell

5 A test was conducted to examine the above-mentioned effect by using sodium salt of N-Acetylneuraminic Acid, in accordance with the same testing method as that of Example 2. The result of this test is shown in Table 7 which shows the following facts.

10 A significant number of rotations was observed upon application of 10^{-3} mg/ml of sodium salt of N-acetylneuraminic acid.

Terbutaline showed a general tendency to increase the rotation at^a/concentration of 10^{-4} to 10^{-5} mg/ml.

15 Acetylcholine showed an increase in the rotation in a certain dependency on the dose, at a concentration of between 10^{-3} and 10^{-2} mg/ml.

20 From these facts, it is understood that the sodium salt of N-acetylneuraminic acid can be used as an expectorant as effectively as N-acetylneuraminic acid and its calcium salt.

Table 7

Effect of Sodium Salt of N-acetylneuraminic Acid on Rotating
Ciliated Explant

Acceleration: mean value \pm standard deviation (%*)

5

10

15

| Drug | Dose (mg/ml) | N | time after perfusion (min) | | |
|---|------------------|---|----------------------------|-----------------|-----------------|
| | | | 5 | 10 | 15 |
| control | - | 8 | 1.0 \pm 5.8 | -0.9 \pm 6.8 | -1.9 \pm 6.8 |
| terbutaline | 10 ⁻⁴ | 3 | -5.2 \pm 12.2 | 1.6 \pm 4.6 | 6.7 \pm 3.0 |
| | 10 ⁻³ | | 51.2 \pm 36.0 | 37.2 \pm 35.5 | 42.1 \pm 32.8 |
| | 10 ⁻² | | 18.6 \pm 0.3 | 24.8 \pm 3.5 | 2.7 \pm 7.4 |
| acetylcholine | 10 ⁻⁴ | 2 | -1.7 \pm 13.4 | 0.8 \pm 12.8 | 14.6 \pm 6.8 |
| | 10 ⁻³ | | 27.8 \pm 12.2 | 10.6 \pm 15.0 | 20.8 \pm 26.4 |
| | 10 ⁻² | | 58.1 \pm 38.1 | 52.0 \pm 32.6 | 44.5 \pm 22.0 |
| sodium salt of N-acetyl- neuraminic acid | 10 ⁻⁴ | 7 | -3.4 \pm 9.7 | 0.6 \pm 10.6 | -5.0 \pm 8.2 |
| | 10 ⁻³ | | 11.6 \pm 29.8 | 20.7 \pm 27.5 | 16.2 \pm 32.3 |
| | 10 ⁻² | | 1.5 \pm 11.6 | 2.1 \pm 18.2 | 3.7 \pm 29.3 |
| | 10 ⁻¹ | | 0.0 \pm 5.8 | 4.7 \pm 6.6 | 15.4 \pm 9.3 |

20 * Acceleration (A%) = $\left(\frac{\text{Cycle in Drug}}{\text{Cycle in Control}} - 1 \right) \times 100$

A > 20.0 is significant.

Example 10:

Effect of Sodium Salt of N-Acetylneuraminic Acid on Sputum

5 The effect of the sodium salt of N-acetylneuraminic acid on sputum was examined in accordance with the testing method explained in connection with Example 5. The result of this test is shown in Table 8.

10 From Fig. 8, it will be seen that the samples treated with the sodium salt of N-acetylneuraminic acid (1 mg/ml sputum) showed a significant increase in pH value and MTR. Although the spinnability was generally increased, a reduction was observed with regard to the yield value.

15 From these facts, it is understood that the sodium salt of N-acetylneuraminic acid can be used effectively as an expectorant which increases the viscosity of low viscosity sputum, such as purulent sputum which is difficult to expectorate, to a high level suitable for transport by cilia, thereby facilitating the expectoration.

Table 8

Rheological properties before and after Sodium Salt of N-Acetylneuraminic Acid Treatment of purulent sputum from patients with diffuse panbronchiolitis

5

10

5

15

| | Treatment | Number of specimens | Mean±S.D. |
|---------------------------------|-----------|---------------------|------------------------|
| MTR (mm/min.) | before | 15 | 9±5 |
| | after | 15 | 12±5 ^a |
| pH | before | 15 | 7.48±0.41 |
| | after | 15 | 7.91±0.43 ^b |
| Yield value, mmH ₂ O | before | 15 | 0.8±1.5 |
| | after | 15 | 0.3±0.6 |
| Spinnability, mm | before | 15 | 13±14 |
| | after | 15 | 21±28 |

a: P < 0.01

b: P < 0.001

Example 11:

20 Acute Toxicity Test of Sodium Salt of N-Acetylneuraminic Acid

25

A test was conducted to examine the acute toxicity of the sodium salt of N-acetylneuraminic acid, in mice, rats, and guinea pig by means of administration per os, subcutaneous injection, intravenous injection, intraperitoneal injection, and inhalation.

(1) Test Animals

ICR mice 6 weeks old
SD rats 6 weeks old
Hartley guinea pig 6 weeks old

5

(2) Application Density

20% (W/V) dissolved in distilled water

(3) Number of Test Animals per Level

10

10

(4) Observation Period

14 days

(5) Method of Calculation of LD₅₀

15

By Probit method

The result of the test is shown in Table 9 below.

Table 9

Acute Toxicity Test of Sodium Salt of N-Acetylneuraminic Acid

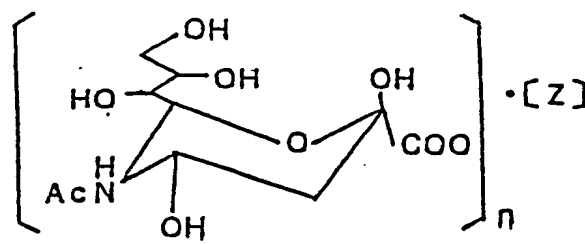
| Species | LD ₅₀ (mg/kg) | | | | |
|------------|--------------------------|------------------------|---------------------------|------------------------|-----------------------------|
| | Way of administration | | | | |
| | per os | subcutaneous injection | intraperitoneal injection | intravenous injection | inhalation |
| mouse | >5,000 | >5,000 | >5,000 | 6,286 (5,679-7,084) | - |
| | >5,000 | >5,000 | >5,000 | 7,951 (7,214-9,073) | - |
| rat | >5,000 | >5,000 | >5,000 | 2,966 (2,638-3,342) | >4,000 mg/m ³ |
| | >5,000 | >5,000 | >5,000 | >5,000 | >4,000 mg/m ³ |
| guinea pig | - | - | - | 2,856 (2,355-4,087) | - |

Inhalation was conducted by 1-hr exposure to mist.

Values in parentheses show 95% reliability limit.

WHAT IS CLAIMED IS

1. An expectorant capable of directly acting on the ciliated cells of the airway and on the rheological property of sputum, comprising a compound of the following formula:



wherein, on condition of $n = 1$, Z represents hydrogen, lithium, potassium, sodium, ammonium, organic ammonium or organic amine, whereas on condition of $n = 2$, Z represents calcium, barium or magnesium, as an effective component.

2. An expectorant according to claim 1 in the form of an aerosol for inhalation.
3. An expectorant according to claim 2 wherein the dose of the effective component is 0.5 to 5 mg/time.

FIG. 1

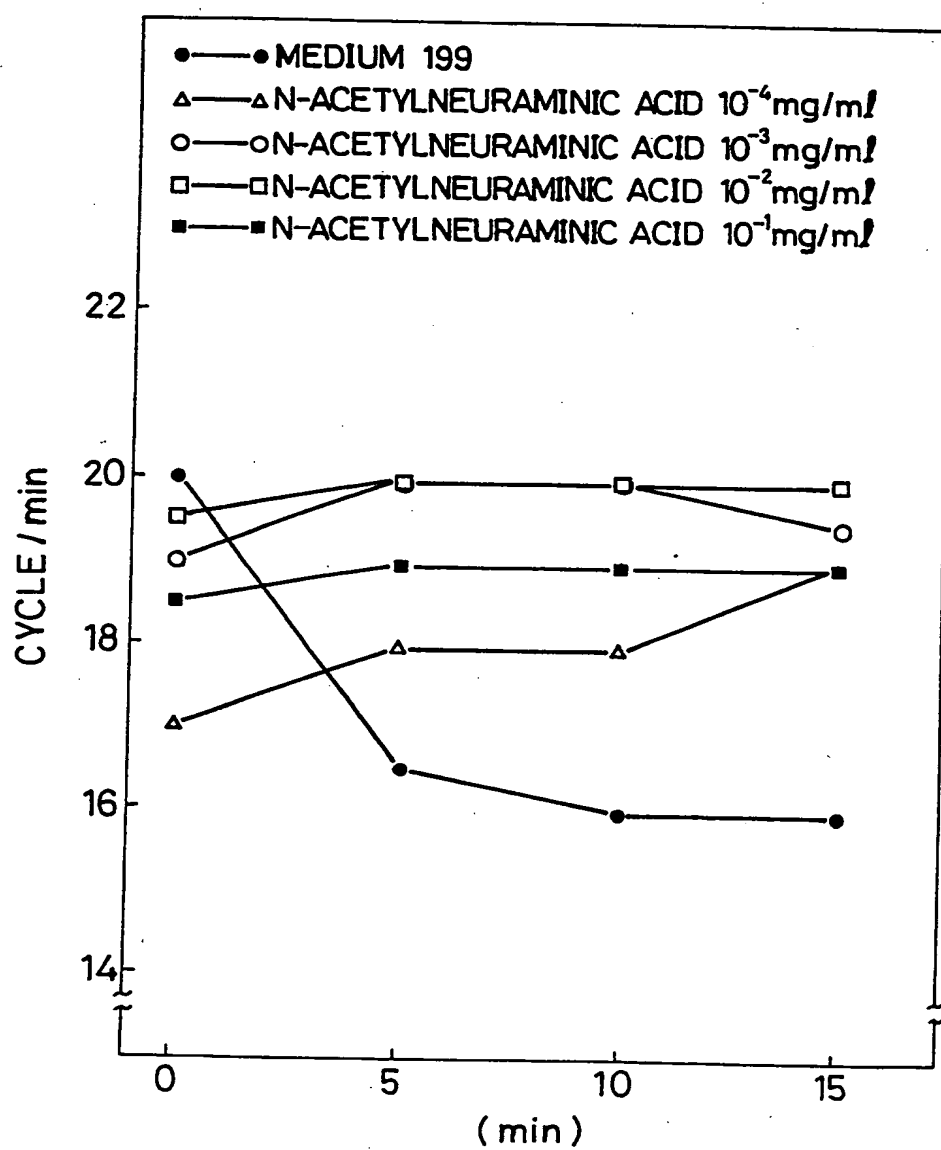
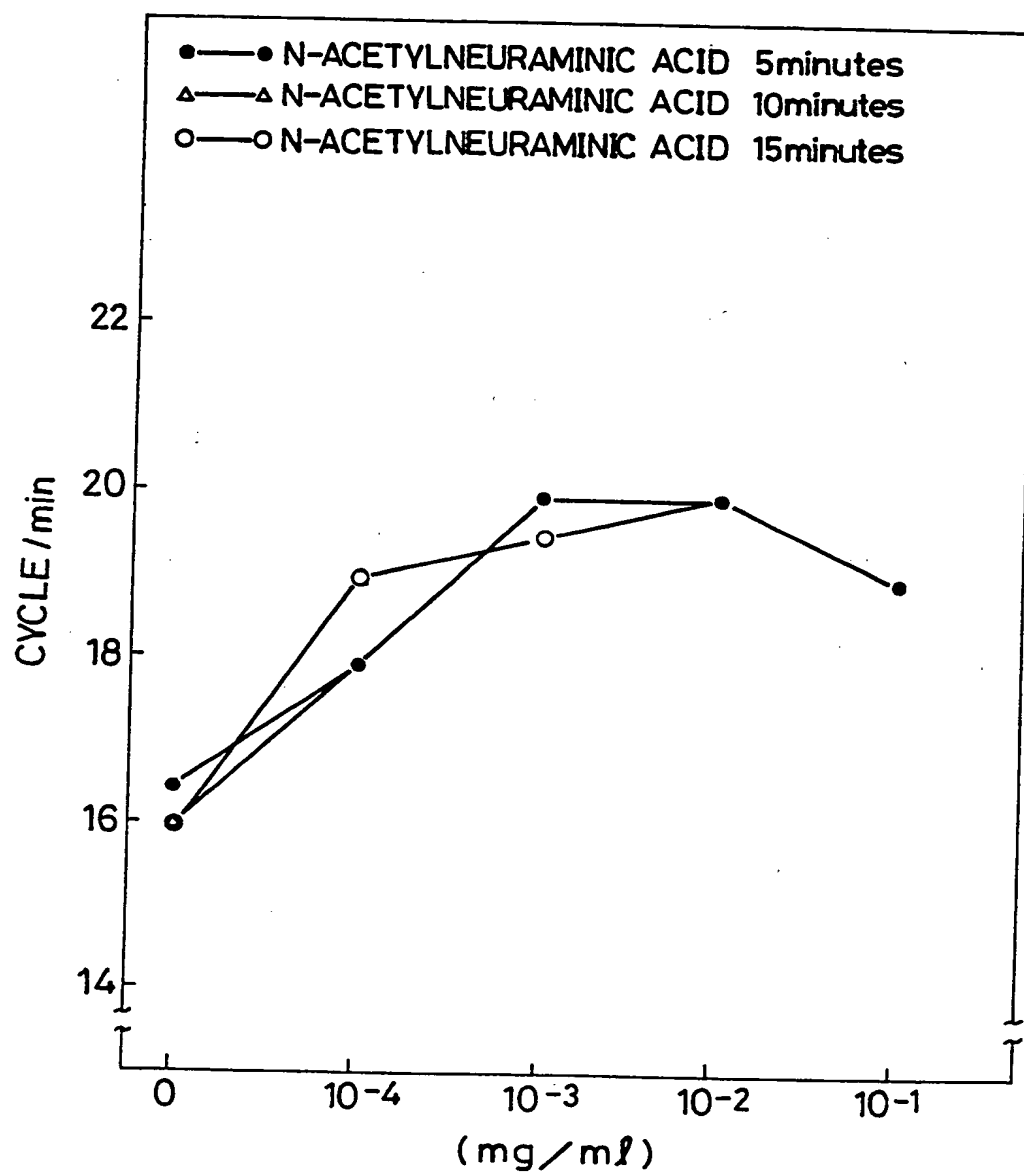


FIG. 2



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FIG. 3

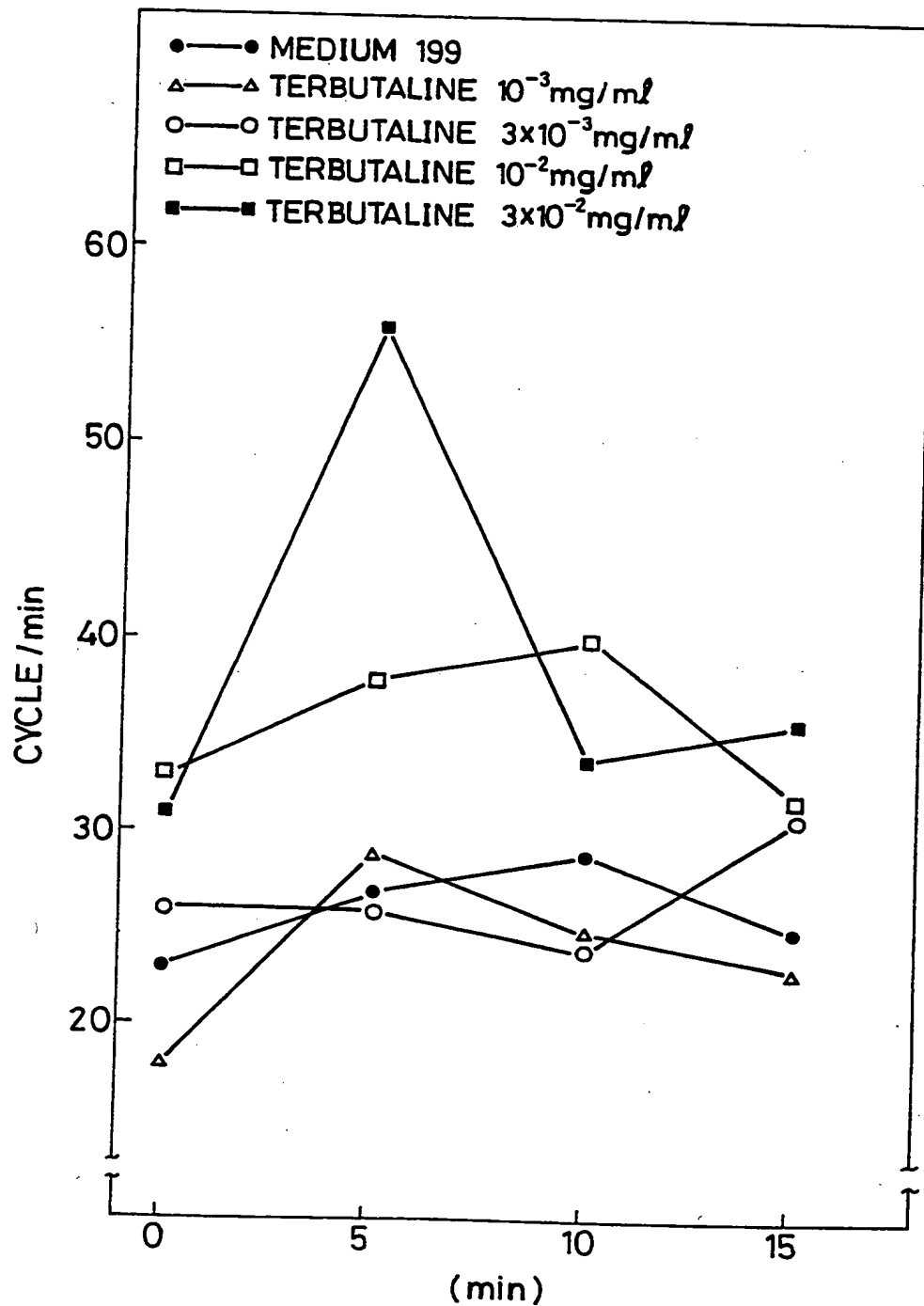


FIG. 4

